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Number of deaths from influenza and pneumonia (all forms) combined—Continued.

City.	Week number.						City.	Week number.					
	First.	Second.	Third.	Fourth.	Fifth.	Sixth.		First.	Second.	Third.	Fourth.	Fifth.	Sixth.
Columbus, Ohio:							Providence, R. I.:						
1922.....	5	9	4	10	8	6	1922.....	13	8	12	17	11	15
1921.....	8	8	12	12	13	12	1921.....	14	6	5	8	14	11
1920.....	15	9	8	22	59	118	1920.....	12	13	8	14	39	88
1919.....	15	14	10	20	19	11	1919.....	47	59	62	61	35	30
Toledo, Ohio:							Nashville, Tenn.:						
1922.....	6	9	8	12	7	6	1922.....	2	7				5
1921.....		3	9	10	5	4	1921.....	2	8	4		10	9
1920.....	9	8	9	18	54	50	1920.....	6	11	6	12	8	23
1919.....	19	15	19	20	15	6	1919.....	20	17	21	21	17	15
Portland, Oreg.:							Richmond, Va.:						
1922.....	4	7	4	6	5	15	1922.....	8	9	9	4	8	9
1921.....	6	5	7	6	4	8	1921.....	5	5	13	6	5	7
1920.....	13	8	9	17	21	57	1920.....	2	9	6	21	35	38
1919.....	55	101	123	122	50	15	1919.....	50	26	34	30	23	11
Philadelphia, Pa.:							Total:						
1922.....	73	98	87	86	85	91	1922.....	671	761	823	863	1,120	1265
1921.....	72	83	85	101	114	108	1921.....	750	737	768	725	738	800
1920.....	55	75	108	153	289	564	1920.....	802	947	1,771	3,820	5,637	5922
1919.....	142	194	229	259	308	262	1919.....	3,165	3,346	3,688	3,756	3,180	2427

THE DETERMINATION OF HYDROGEN ION CONCENTRATION.

By FRANCIS H. McCrudden, Surgeon (R), United States Public Health Service; United States Veterans' Hospital No. 36, Boston, Mass.

The acidity of a solution may be expressed in terms of concentration of acid or in terms of hydrogen ion concentration. Though the significance and relationship of these two modes of expression and the practical determination of degree of acidity in both senses have become of considerable practical importance in medicine, I have not yet seen a statement of the subject simple enough to enable the physician with very little knowledge of chemistry to understand and actually determine hydrogen ion concentration. The statement which follows, framed especially for the bacteriologist with very little knowledge of chemistry, is intended as a step in this direction.

1. CONCENTRATION OF ACID.

Normal solutions.—The concentration of acid solutions is not usually expressed in percentage strength but as normal (N), half normal ($\frac{N}{2}$, 0.5 N), tenth normal ($\frac{N}{10}$, 0.1 N), etc. A normal solution of acid is one containing one gram of hydrogen per liter replaceable by a base. This chemical use of the term "normal" with reference to acids and alkalis is not to be confused with the use of the term in the expression "normal salt solution" where it means a solution normal to, or containing as much salt as blood and tissue fluids—about three-quarters of 1 per cent.

Examples: A normal solution of hydrochloric acid contains 36.5 grams HCl per liter (atomic weights: H = 1, Cl = 35.5). A normal solution of sulphuric acid (H_2SO_4), which has two replaceable hydrogens in the molecule, contains 49 grams, half a molecular weight, per liter (atomic weights: H = 1, S = 32, O = 16). Crystalline oxalic acid has two replaceable hydrogens and two molecules of water of crystallization ($\text{C}_2\text{O}_4\text{H}_2 \cdot 2 \text{H}_2\text{O}$; molecular weight, 126); a normal solution contains 63 grams oxalic acid. The formula of acetic acid is $\text{CH}_3 \text{COOH}$ (molecular weight, 60), and it has only one hydrogen atom replaceable by base; a normal solution contains 60 grams pure acetic acid. In determining the amount of acid to be used in making up a normal solution we must consider whether the acid is monobasic, like hydrochloric acid, or dibasic, like sulphuric acid; we must consider its whole molecular weight, including any water of crystallization present as in the case of oxalic acid; and we must consider whether, as in the case of acetic acid and most other organic acids, some of the hydrogen is not replaceable by base.

Experiments on equivalence of normal solution.—Make 500 c. c. of approximately $\frac{N}{10}$ hydrochloric acid, sulphuric acid, oxalic acid, acetic acid, and sodium hydroxide as follows:

Hydrochloric acid: Make 4.9 c. c. of concentrated hydrochloric acid (U. S. P. specific gravity 1.155, containing about 32 per cent HCl) up to 500 c. c.

Sulphuric acid: Make 1.42 c. c. of concentrated sulphuric acid (U. S. P. specific gravity 1.83, containing about 94 per cent $\text{H}_2 \text{SO}_4$) up to 500 c. c.

Oxalic acid: Dissolve 3.150 grams $\text{C}_2\text{H}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$ in 500 c. c. water.

Acetic acid: Make 2.9 c. c. glacial acetic acid (U. S. P. specific gravity 1.048, containing 99 per cent $\text{HC}_2\text{H}_3\text{O}_2$) up to 500 c. c.

Sodium hydroxide: Dissolve 2 grams of sodium hydroxide in 500 c. c. water.

Measure out 10 c. c. of each of the acid solutions into small flasks and to each add two drops of 1-2 per cent alcoholic solution of phenolphthalein. Run $\frac{N}{10}$ sodium hydroxide drop by drop into each flask until the solution just turns pink.

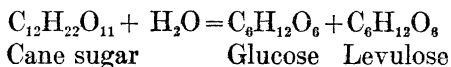
Oxalic acid practically 100 per cent pure, from which accurately $\frac{N}{10}$ oxalic acid may be prepared, is readily obtained. The other solutions can be made only approximately $\frac{N}{10}$ at first; but after comparison with the accurate $\frac{N}{10}$ oxalic acid, they can be corrected by dilution with water or addition of acid or alkali.

Normality and percentage strength.—Since equal volumes of all normal acids will neutralize equal volumes of all normal alkali solutions, the use of acids and bases in the form of normal or fractionally normal solutions, rather than in percentage strength, is of practical convenience in simplifying calculations.

Measurement of acid concentration.—The concentration of acid in a solution may be determined by titration with standard alkali solution.

2. STRENGTH OF ACIDS.

A liter of normal acetic acid will neutralize the same amount of alkali as a liter of normal hydrochloric acid. In this respect the "acidity" of a liter of normal acetic acid is the same as that of a liter of normal hydrochloric acid. But acid solutions have other properties besides that of neutralizing alkalis which are proportional to the "acidity" of the solution and can, therefore, serve to measure "acidity." If a solution of cane sugar is boiled with acid, the sugar breaks down into one molecule of glucose and one of levulose:



The acid does not appear to take a direct part in the reaction; it acts as a stimulator to hasten the reaction, the rate at which the reaction takes place depending on the concentration of the acid. A tenth normal hydrochloric acid solution has twice as great a stimulating effect as a twentieth normal, and half as great an effect as a fifth normal solution. And a tenth normal acetic acid solution has twice as great an effect as a twentieth normal, and half as great an effect as a fifth normal solution. *But* a tenth normal solution of hydrochloric acid has a far greater effect as a stimulator than a tenth normal solution of acetic acid. In this respect the "acidity" of the two tenth-normal solutions is not the same. Hydrochloric acid is a "stronger" acid than acetic acid. To understand the apparent contradiction in the two different senses of the word "acidity", it is necessary to understand something about the dissociation theory.

Experiments on strength of acid.—Make an $\frac{M}{2}$ (half molecular) solution of cane sugar (dissolve 17.1 grams of sugar and make up to 100 c.c.).

Prepare the following solutions in test tubes:

(a) 1 c.c. $\frac{M}{2}$ cane sugar + 1 c.c. $\frac{N}{10}$ HCl + 1 c.c. water.

(b) 1 c.c. $\frac{M}{2}$ cane sugar + 0.5 c.c. $\frac{N}{10}$ HCl + 1.5 c.c. water.

(c) 1 c.c. $\frac{M}{2}$ cane sugar + 3.0 c.c. $\frac{N}{10}$ acetic acid.

(d) 1 c.c. $\frac{M}{2}$ cane sugar + 2 c.c. water.

Boil each of these solutions exactly one minute. Cool. Dilute to 25 c.c. Add each solution drop by drop from a buret to a boiling Fehling's solution (4 c.c. of the copper solution and 6 c.c. of the alkaline tartrate) until the Fehling's solution is decolorized (in test (c) the Fehling's will still remain blue after all the sugar has been added, showing that less than 10 per cent of the sugar has been hydrolyzed). Calculate the amount of reducing sugar present, and from this the percentage of cane sugar hydrolyzed in cases (a) and (b).

3. THE DISSOCIATION THEORY.

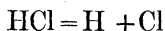
Molecules in solution.—It has long been known that certain properties of a solvent are modified by substances in solution. An aqueous solution of cane sugar, for example, has a higher boiling point, a lower freezing point, and a lower vapor pressure than pure water, and it exerts an osmotic pressure. The rise in boiling point, the fall in freezing point, the diminution in vapor pressure, and the osmotic pressure are proportional to the amount of sugar in solution. The same is true for aqueous solutions of other substances—urea, for example. The effect of one gram molecule (60 grams) of urea ($\text{CO}(\text{NH}_2)_2$) on these properties of the solutions is precisely the same as that of one gram molecule (342 grams) of cane sugar ($\text{C}_{12}\text{H}_{22}\text{O}_{11}$) or one gram molecule of any other substance. All this indicates that the effect of substances in solution on the properties of the solution depends on the number of molecular weights of the substance per unit volume of solution; or, translated into terms of the molecular hypothesis, the number of dissolved molecules per unit volume.

Ions in solution.—There are apparent exceptions to the foregoing. It holds true for sugar, urea, and most other organic substances; but the effect of salts, strong acids, and bases is much greater. Sodium chloride, hydrochloric acid, sodium hydroxide, for example, in dilute solution have just twice as great an effect, and sodium sulphate three times as great an effect as they should. The deviations are due to the dissociation of these substances into ions. Sodium chloride (NaCl) is dissociated into a positive ion, or cation, Na , and a negative ion, or anion, Cl ; hydrochloric acid (HCl) is dissociated into the cation H and the anion Cl ; sodium hydroxide (NaOH) into the cation Na and the anion OH ; sodium sulphate (Na_2SO_4) into two Na cations and the anion SO_4 . The quantitative effect of these ions on the physical properties of solutions is the same as that of whole undissociated molecules.

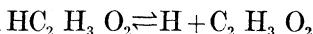
Electrolytes.—Solutions of sodium chloride, hydrochloric acid, sodium hydroxide, and other substances which dissociate, transmit the electric current; such compounds are called electrolytes. The current is transmitted by the moving ions; each cation carries a positive charge, each anion a negative charge of electricity. When an electric current is passed through a solution of silver nitrate, for example, the Ag cations pass with the current to the cathode, give up their positive charge, and are deposited as molecular silver; the NO_3 anions pass to the anode and are there discharged. Solutions of urea, cane sugar, and other substances which do not dissociate, do not transmit the electric current; such substances are called non-electrolytes. Besides the electrolytes like sodium chloride which are completely dissociated in dilute solution, and the non-electrolytes

like urea which are not dissociated, there are substances like acetic acid and ammonia which are slightly dissociated; these are called weak electrolytes. Speaking generally, the electrolytes include all salts, strong acids, and bases; the weak electrolytes include the weak acids and weak bases; the non-electrolytes include organic substances which are not salts, acids, or bases.

Dissociation constant.—We can write the reaction for dissociation of the strong electrolyte hydrochloric acid as follows:



In dilute solution the reaction is complete from left to right; there is no undissociated HCl in solution. The reaction for the weak electrolyte acetic acid would be written as follows:



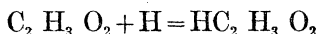
The sign of reversibility \rightleftharpoons , instead of equality, $=$, indicates that the reaction goes in both directions, that when equilibrium is reached, there is always some of all three of the reacting substances—undissociated acetic acid, hydrogen ion, and the acetic acid anion—present. The extent of dissociation—that is, the amount of each of the reacting substances present when equilibrium is reached—is determined by a law, the mass law, which all reversible reactions are found to obey, according to which the extent of reaction is proportional to the mass of the reacting substances. According to the mass law, the product of the concentration of the ions bears a constant ratio to the concentration of the undissociated substance, a relationship which is expressed as follows:

$$K = \frac{c_1 \times c_2}{C}$$

In the case of acetic acid, c_1 is the concentration of hydrogen ions, c_2 the concentration of the $\text{C}_2\text{H}_3\text{O}_2$ ions, and C the concentration of the undissociated acetic acid. K is a constant—in this case, 0.000018—the dissociation constant for acetic acid.

Dissociation of acetic acid.—Using 0.0001 (a rough approximation that will simplify presentation) for the dissociation constant of acetic acid, $\frac{c_1 \times c_2}{C} = 0.0001$, or $c_1 \times c_2 = 0.0001 C$. That is to say, in a dilute solution of acetic acid, the product of the concentration of the hydrogen ions and the acetic acid anion is one ten-thousandth that of the concentration of the undissociated acetic acid. In a normal solution of acetic acid, $C = 1$, and $c_1 \times c_2 = 0.0001$; if the solution contains no other acid and no acetate, $c_1 = c_2 = 0.01$. According to this calculation, a normal solution of acetic acid is about one per cent dissociated. (The correct figure is $\sqrt{0.000018}$, or about 0.4 per cent.)

Diminution of dissociation.—In the equation for acetic acid, $K = \frac{c_1 \times c_2}{C}$, or $c_1 \times c_2 = KC$, C , the undissociated acetic acid, is so nearly constant (it varies from 99.5 to 100 per cent of the total acetic acid in a dilute solution) that we may call it constant, and write $c_1 \times c_2 = K_1$, where $K_1 = KC$. If now, in a solution of acetic acid, we increase c_2 by adding $C_2 H_3 O_2$ anion in the form of the completely dissociated sodium acetate, then, in order to maintain K_1 constant, c_1 must decrease. The following reaction takes place:



In other words, the addition to an acid solution of a salt of the same acid diminishes the dissociation of the acid, diminishes the "acidity," diminishes the hydrogen ion concentration.

Calculation of hydrogen ion concentration.—The equation $c_1 \times c_2 = KC$ may be written $c_1 = K \frac{C}{c_2}$. In a solution containing a weak acid and a salt of the acid, C , the concentration of the undissociated acid, is practically the total concentration of the acid, dissociated and undissociated; and, since all of the salt is dissociated and very little of the acid, c_2 , the concentration of the anion, is practically the concentration of the salt. We may, therefore, write—

$$\text{concentration of hydrogen ions} = K \frac{\text{concentration of acid}}{\text{concentration of salt}}$$

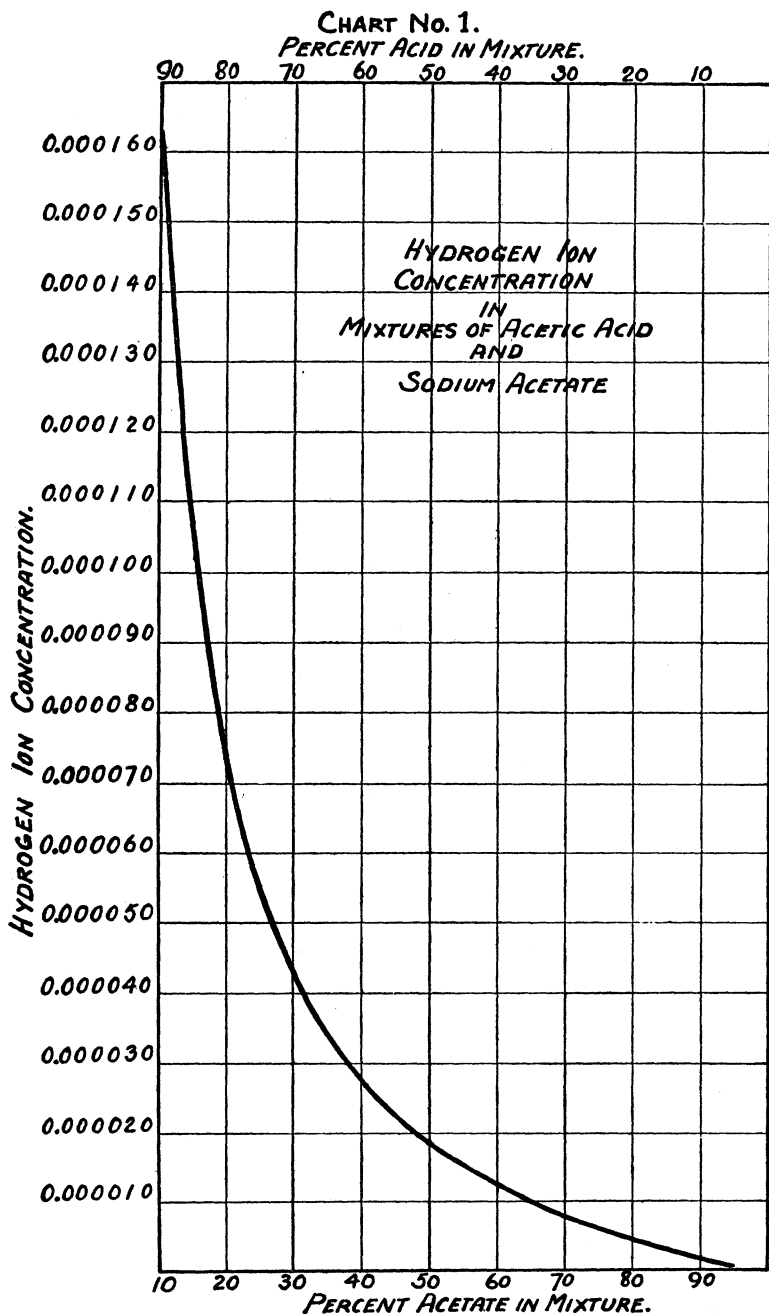
Table I and Chart No. 1 show the hydrogen ion concentration in mixtures of acetic acid and sodium acetate.

TABLE I.

c_2 Parts acetate in 10	C Parts acetic acid in 10	Hydrogen ion con- centration. $K \frac{C}{c_2} = c_1$
1	9	$0.000018 \times \frac{9}{1} = 0.000162$
2	8	$0.000018 \times \frac{8}{2} = 0.000072$
3	7	$0.000018 \times \frac{7}{3} = 0.000042$
4	6	$0.000018 \times \frac{6}{4} = 0.000027$
5	5	$0.000018 \times \frac{5}{5} = 0.000018$
6	4	$0.000018 \times \frac{4}{6} = 0.000012$
7	3	$0.000018 \times \frac{3}{7} = 0.000008$
8	2	$0.000018 \times \frac{2}{8} = 0.000004$
9	1	$0.000018 \times \frac{1}{9} = 0.000002$

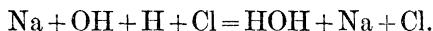
The figures indicate how great a reduction in the acidity of a weak acid results from addition of comparatively small amounts of a salt of the same acid; an increase of the proportion of salt from ten up to 20 per cent in the mixture diminishes the acidity by half, and a further increase of ten per cent reduces the acidity again by a half. The figures show, further, that the hydrogen ion concentration in a solution containing a mixture of weak acid and salt of the same acid in

equal proportions is numerically equivalent to the dissociation constant; when $C=c_2$, then the equation $c_1 = K \frac{C}{c_2}$ becomes $c_1 = K$.



Dissociation of water.—Water dissociates to a very slight extent, so slight that $C_{\text{H}_2\text{O}}$ in the equation $C_{\text{H}} \times C_{\text{OH}} = K C_{\text{H}_2\text{O}}$ may be regarded

as a constant, and we may write $C_H \times C_{OH} = K$. K is 0.00000000000001 or 10^{-14} . In pure water, $C_H = C_{OH} = 10^{-7}$. The degree of dissociation of water is so slight that for most practical purposes water may be regarded as undissociated. It is because water dissociates so little that acids and bases react in solution to form salts. Before the publication of the dissociation theory, the cause of the reaction $HCl + NaOH = NaCl + HOH$ was seen in the attraction or affinity between sodium and chlorine. Now we write the reaction—



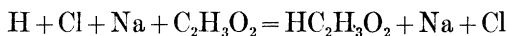
What really happens is that the hydrogen ions from the acids and the hydroxyl ions from the base unite to form undissociated HOH ; the sodium and chloride ions remain unaffected. In the case of a weak, slightly dissociated acid like acetic acid, the result is the same; as fast as the free hydrogen ions disappear to form undissociated HOH , more hydrogen ions are set free from the undissociated weak acid, until finally all the acid, dissociated and undissociated, has been converted to salt. Titration with alkali measures the total concentration of acid, dissociated and undissociated; it does not measure the hydrogen ion concentration.

p_H .—The hydrogen ion concentration of a solution containing equal parts acetic acid and acetate can be expressed in the following different ways: $0.000018 = \frac{18}{1,000,000} = \frac{1.8}{100,000} = \frac{1.8}{10^5} = 1.8 \times \frac{1}{10^5} = 1.8 \times 10^{-5} = 10^{0.25} \times 10^{-5} = 10^{-4.75}$. The simplest of these figures for defining the hydrogen ion concentration is the negative logarithm 4.75; and so it is everywhere used for this purpose instead of the more cumbersome figures. It is referred to as p_H . p_H is likewise more convenient to plot on charts than C_H . The change in C_H from normal acid to pure water runs from 1.0 to 0.0000001. On a scale where the change from 0.0000001 to 0.000001 would be one inch, the change from 0.0000001 to 1.0 would be one million inches or several miles. The corresponding variation in p_H runs from 7 to 0, and can be plotted on a scale seven inches long. p_H can be used to define the alkalinity as well as acidity. In a $\frac{N}{100}$ alkaline solution, for example, $C_{OH} = \frac{1}{100}$ or 10^{-2} . Then, since $C_H \times 10^{-2} = 10^{-14}$, $C_H = 10^{-12}$, and $p_H = 12$. Chart No. 2 shows the p_H value for mixtures of acetic acid and sodium acetate. (p_H in this case is the logarithm of c_1 , Table 1.)

4. BUFFER MIXTURES.

Definition.—From Chart No. 2 it will be seen that a solution containing acetic acid and sodium acetate in any except extreme proportions has a fairly constant acidity. With equal parts acid

and salt, p_H is 4.75; with three parts acid and seven parts salt p_H is 5.1; with seven parts acid and three parts salt, p_H is 4.4. Now if we add to a mixture containing equal parts acid and salt, alkali enough to convert some of the acid to salt, we merely change the proportion of acid and salt from 5:5, let us say, to 3:7, a change which does not greatly affect p_H . If we add a strong, completely dissociated acid like hydrochloric acid, the highly concentrated hydrogen ions from this acid will unite with the acetic acid anions from the sodium acetate to form undissociated acetic acid:



The end result will be a decrease in the sodium acetate, and an increase in the acetic acid, the proportion of salt to acid changing from 5:5, let us say, to 3:7—a change which does not greatly affect p_H . Mixtures of this kind (mixtures of a weak acid and a salt of the acid) to which either alkali or acid may be added in moderate quantities with but little change in p_H are called buffer mixtures.

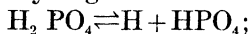
Experiments on buffer mixtures—Acetic acid and sodium acetate.—Observe the color imparted to dilute acid, to distilled water and to dilute alkali by two drops of a 1–2 per cent alcoholic solution of dimethylamidoazobenzol, and by two drops of 1–2 per cent alcoholic solution of phenolphthalein. Add both indicators together to 10 c. c. of water, and determine how many drops of $\frac{N}{10}$ HCl is required to turn this neutral solution red (acid); how many drops of $\frac{N}{10}$ alkali is required to turn this neutral solution red (alkaline). Now mix together 5 c. c. $\frac{N}{10}$ acetate acid and 5 c. c. $\frac{N}{10}$ sodium acetate solution, add two drops of each of these indicators and determine how much $\frac{N}{10}$ hydrochloric acid can be added before this neutral solution becomes acid. How much $\frac{N}{10}$ sodium hydroxide is required to turn such a mixture alkaline.

Important buffer mixtures.—Two buffer mixtures of great practical importance in physiology are the phosphate pair, $NaH_2PO_4 : Na_2HPO_4$, and the carbonate pair, $H_2CO_3 : NaHCO_3$.

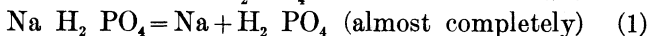
NaH_2PO_4 .—Phosphoric acid, H_3PO_4 , has three hydrogens replaceable by base. H_3PO_4 is a strong acid dissociated in solution as follows:



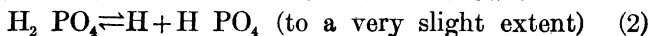
The anion H_2PO_4 is a very weak acid, far weaker than acetic acid, which dissociates to a very slight extent as follows:



Its dissociation constant is about 10^{-7} . HPO_4 does not further dissociate. The salt NaH_2PO_4 dissociates as follows:

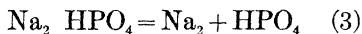


and then the anion further dissociates as follows:

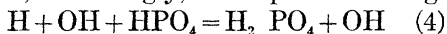


As a result of this second reaction a solution of $\text{Na H}_2\text{PO}_4$ reacts weakly acid.

Na_2HPO_4 .—This salt dissociates in solution as follows:



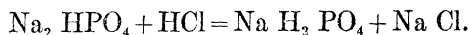
Now the dissociation constant of H_2PO_4 is so small, so near that of pure water, that even the small quantities of hydrogen ion resulting from the dissociation of water can not remain in the presence of the large amount of HPO_4 ion coming from Na_2HPO_4 . The following reaction, accordingly, takes place to a slight extent:



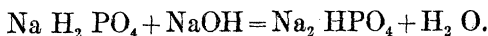
leaving a slight excess of the alkaline hydroxyl ions in the solution.

The $\text{Na H}_2\text{PO}_4$: Na_2HPO_4 mixture.—Reactions (2) and (4) are reversible reactions; addition of any of the members appearing on the right hand side of the equation makes the reactions run from right to left. Addition of even a small quantity of Na_2HPO_4 , which gives much HPO_4 , to a solution of $\text{Na H}_2\text{PO}_4$ makes reaction (2) run from right to left; that is to say, it diminishes the acidity of $\text{Na H}_2\text{PO}_4$ to practically the neutral point. Similarly, addition of even a small amount of $\text{Na H}_2\text{PO}_4$, which gives much H_2PO_4 , to a solution of Na_2HPO_4 makes reaction (4) run from right to left; that is, it diminishes the alkalinity of Na_2HPO_4 to practically the neutral point. Accordingly, a solution containing a mixture of NaH_2PO_4 and Na_2HPO_4 in any proportions except extreme ones is almost precisely neutral.

Na_2HPO_4 : $\text{Na H}_2\text{PO}_4$ as a buffer mixture.—If, now, to a solution containing, say, equal parts $\text{Na H}_2\text{PO}_4$ and Na_2HPO_4 we add HCl , some of the alkaline phosphate is converted to acid phosphate:

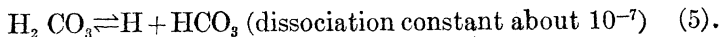


If instead of HCl we add NaOH , some of the acid phosphate is converted to alkaline phosphate:

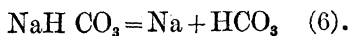


The end result of adding either acid or alkali to such a mixture is merely a change in the proportion of the two salts present; and since a mixture of the two salts in any except extreme proportions is almost precisely neutral, these two salts form a buffer mixture.

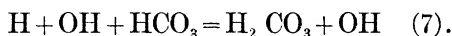
H_2CO_3 : Na HCO_3 buffer mixture.—The weak acid H_2CO_3 dissociates to a slight extent as follows:



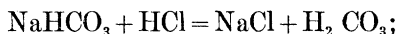
The salt NaHCO_3 dissociates completely as follows:



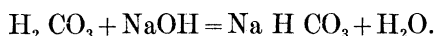
Even the slight quantity of hydrogen ions from the dissociation of water reacts with the HCO_3 :



$\text{H}_2 \text{CO}_3$ is, therefore, a very weak acid (reaction 5); and NaHCO_3 is a very weak alkali (reaction 7). But addition of even a very small amount of NaHCO_3 to a solution of carbonic acid diminishes the acidity to practically the neutral point (reaction 5 goes from right to left) and addition of even a little $\text{H}_2 \text{CO}_3$ to a solution of NaHCO_3 diminishes its alkalinity to practically the neutral point (reaction 7 goes from right to left). In other words, a mixture of carbonic acid and sodium bicarbonate in any except extreme proportions is almost precisely neutral. Addition of acid to such a mixture changes some of the bicarbonate to acid:



and addition of alkali changes some of the acid to bicarbonate



The change in p_{H} resulting from the change in relative amounts of acid and salt is very little.

p_{H} of these buffer mixtures.—In the equation $\frac{C_{\text{H}} \times C_{\text{HPO}_4}}{C_{\text{H}_2 \text{PO}_4}} = K$,

K is 1.55×10^{-7} . Then $C_{\text{H}} = 1.55 \times 10^{-7} \times \frac{C_{\text{H}_2 \text{PO}_4}}{C_{\text{HPO}_4}}$. In a mixture of these two phosphates the concentration of $\text{H}_2 \text{PO}_4$ is practically that of $\text{NaH}_2 \text{PO}_4$, and the concentration of HPO_4 practically that of the $\text{Na}_2 \text{HPO}_4$. So we may write:

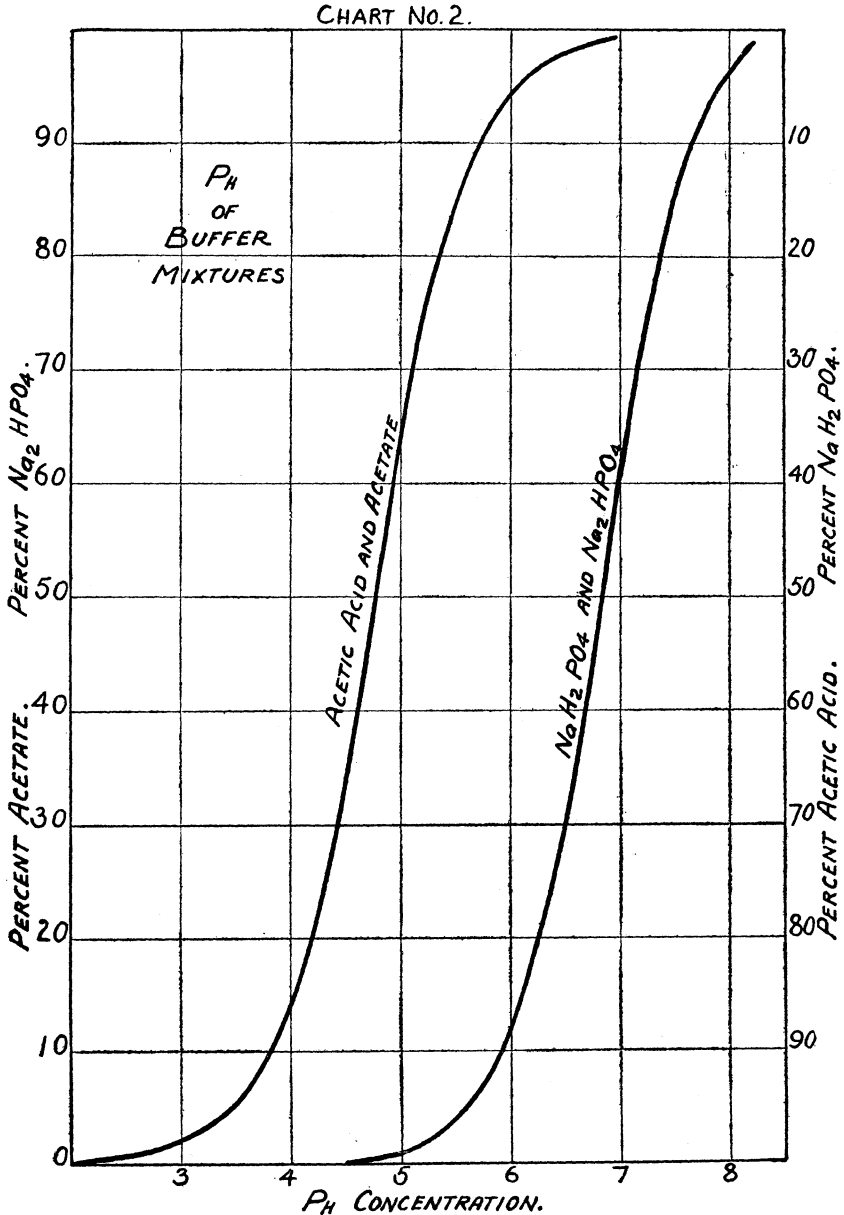
$$C_{\text{H}} = 1.55 \times 10^{-7} \times \frac{\text{concentration of acid phosphate}}{\text{concentration of alkaline phosphate}}.$$

Table II and Chart 2 show the hydrogen ion concentration of mixtures of the two phosphates.

TABLE II.— p_{H} of mixture of $\text{Na}_2 \text{HPO}_4$ and $\text{NaH}_2 \text{PO}_4$.

Percentage $\text{NaH}_2 \text{PO}_4$ in mixture.	Percentage $\text{Na}_2 \text{HPO}_4$ in mixture.	C_{H} .	p_{H} .
10	90	0.17×10^{-7}	7.77
20	80	0.38×10^{-7}	7.42
30	70	0.66×10^{-7}	7.18
40	60	1.03×10^{-7}	6.99
50	50	1.55×10^{-7}	6.81
60	40	2.32×10^{-7}	6.63
70	30	3.13×10^{-7}	6.50
80	20	6.2×10^{-7}	6.21
90	10	14×10^{-7}	5.85

From Table II and Chart 2 it will be seen that p_H in mixtures of alkaline and acid phosphate lies between the limits 6 and 8; this means a variation in reaction of only from $\frac{N}{1,000,000}$ acid to $\frac{N}{1,000,000}$ alkali. The dissociation constant for H_2CO_3 is practically the same as for H_2PO_4 , so that Table II and Chart 2 are practically correct for the $H_2CO_3:NaHCO_3$ buffer mixture.



Their physiological importance.—These two pairs of buffer mixtures occur in blood and tissue fluids and maintain the reaction of the body fluids precisely neutral. The end products of metabolism are more acid than alkaline, so that there would be a tendency to accumulation of the acid members of these buffer mixtures were this tendency not combated by the continuous excretion of the excess of these acid members, the acid phosphate through the kidneys, the carbonic acid through the lungs.

5. COLORIMETRIC DETERMINATION OF HYDROGEN ION CONCENTRATION.

Nature of indicators.—The indicators used in determining the reaction of a solution are weak organic acids whose anion has a different color in solution from that of the undissociated acid. Methyl orange, for example, is pink in acid solution and yellow in alkaline solution. In alkaline solution the indicator is all present as salt; the salt is completely dissociated, and the anion gives its characteristic yellow color to the solution. In acid solution, the excess of hydrogen ions diminishes the dissociation of the weakly acid indicator just as hydrochloric acid diminishes the dissociation of acetic acid; and the undissociated acid gives its characteristic pink color to the solution.

p_H and color of indicator.—The p_H of mixtures of the pink undissociated acid methyl orange and the yellow salt of methyl orange varies slightly about a mean just as does the p_H of buffer mixtures; the mean value, as in the case of buffer mixtures, is numerically equivalent to the dissociation constant. The form of the curve for p_H is similar to that of the curves for p_H in mixtures of acetic acid and sodium acetate and other buffers. Since, now, mixtures of the acid methyl orange and the methyl orange salt in varying proportions correspond to definite hydrogen ion concentrations; and since these mixtures show varying shades of orange likewise corresponding to the proportions of the pink acid solution and the yellow salt solution, each shade of orange corresponds to a definite hydrogen ion concentration. And this fact gives the basis for a colorimetric method of determining hydrogen ion concentration.

Indicators useful in bacteriology.—Any one indicator changes color over only a very small range of hydrogen ion concentration; and any one buffer mixture acts as such over only a very small range of hydrogen ion concentration. To cover a large range would require, therefore, a considerable number of indicators and several pairs of buffer mixtures. In bacteriological work we are interested chiefly in values for p_H close to neutrality ($p_H=7$), a region which is covered by the phosphate buffer mixture ($p_H=5.8$ to 8.2). There are several indicators for this region; brom cresol purple, which shows changes from yellow to purple between p_H 5.2 and p_H 6.8, and phenol

red, which shows color changes from yellow to red between p_H 6.8 and p_H 8.4, are among the best.

6. TECHNIQUE OF THE DETERMINATION.

The standard solutions.—Tenth molecular solutions of KH_2PO_4 (13.62 grams potassium phosphate, monobasic, anhydrous, Merck's reagent, to the liter) and Na_2HPO_4 (14.21 grams sodium phosphate, anhydrous, Merck's reagent, per liter) are convenient strengths for the stock solutions. From these the following twelve standard solutions are prepared:

TABLE III.— p_H of phosphate solutions.

C. c. $\frac{M}{10}$ Na_2HPO_4	C. c. $\frac{M}{10}$ KH_2PO_4	p_H
8	92	5.8
12	88	6.0
19	81	6.2
27	73	6.4
37	63	6.6
49	51	6.8
61	39	7.0
73	27	7.2
82	18	7.4
89	11	7.6
94	6	7.8
97	3	8.0

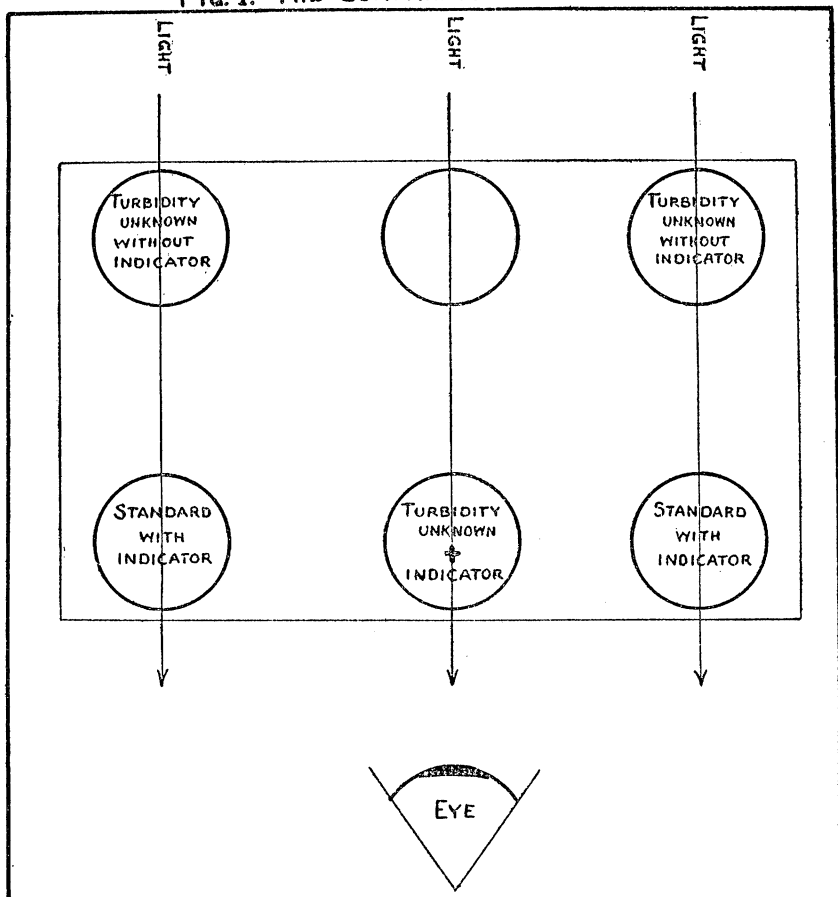
The reading.—To determine the hydrogen ion concentration of an unknown solution coming within the limits of $p_H=6.8$ to 8.2, add to it five drops of a 0.03 per cent solution of phenol red and compare the resulting color with that obtained by adding the same amount of indicator to 5 c. c. of each of the standard phosphate solutions diluted with 10 c. c. of water. Between the limits $p_H=5.8$ to 6.8 the indicator brom cresol purple—five drops of a saturated solution—should be used. (The standard solutions with indicator in them will keep several weeks if tightly stoppered.)

The comparator.—The color comparison can be made in large clear glass test tubes. To overcome the effect of turbidity, such as occurs in bacteriological media, the unknown solution is diluted to a moderate extent, say to three times its volume, and the test tubes are arranged in a device called a comparator. The device consists of a block of wood containing 6 perpendicular holes large enough to carry the test tubes. Three other holes are then bored horizontally through the block from side to side, so that one can look right through each pair of test tubes in series. When the solutions are arranged as indicated in Figure 1, in each case the light reaching the eye has passed through solution containing indicator and solution containing turbidity. In the case of the unknown, one solution contains both

turbidity and indicator; in the case of the standards the turbidity and indicator are in separate solutions.

Adjusting reaction of culture media.—Most bacteria grow best in media whose p_H lies between 7.2 and 7.6. The optimum for typhoid and paratyphoid is near the lower of these two limits; that for pneumococcus, streptococcus, and meningococcus, nearer the upper of

FIG. 1:— THE COMPARATOR.



these limits. To adjust media to any desired hydrogen ion concentration, $\frac{N}{10}$ alkali is added drop by drop to five c. c. of the somewhat diluted media containing indicator until, as shown by comparison with the standards, the desired hydrogen ion concentration is reached. From the amount of alkali required for five c. c., the amount needed for the whole batch of media can then be calculated. Sterilization of the media shifts the p_H about 0.2 toward the acid side. Allowance should be made for this.